

## HUBUNGAN POLIMORFISME GEN RESEPTOR ESTROGEN ALFA DENGAN JUMLAH SEL T CD4+ PADA ANAK TERINFEKSI HIV

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### Keywords:

Children, HIV, ER $\alpha$  polymorphism, PvuII, XbaI

### ABSTRACT

**Background:** Estrogen plays a key role in human physiological processes. Polymorphisms of estrogen receptors have been implicated in the development of numerous diseases. The aim of this study was to evaluate the frequency of ER $\alpha$  gene PvuII and XbaI polymorphisms and assessing their association with CD4+ T-cell counts in HIV-infected children on highly active antiretroviral therapy.

**Methods:** CD4+ T cell counts were determined using the FACS count system. ER $\alpha$  PvuII and XbaI polymorphisms were analyzed by PCR-RFLP.

**Results:** This study enrolled 34 HIV-infected children on HAART. The frequencies of the PvuII and XbaI gene polymorphisms were PP 41,2%, Pp 26,5%, pp 32,4% and XX 35,3%, Xx 17,6%, xx 47,1% respectively. CD4+ T-cell counts were significantly associated with XbaI polymorphisms ( $p < 0.05$ ), but not PvuII polymorphisms ( $p > 0.01$ ).

**Discussion:** Host genetic factor polymorphism is an important determinant of HIV disease progression and treatment response. The ER $\alpha$  PvuII and XbaI polymorphisms can increase risk for the development of HIV-related complication, including oral diseases.

**Conclusion:** The ER $\alpha$  gene XbaI polymorphism was significantly associated with CD4+ T-cell counts. It may explain the role of estrogen in the regulation of HIV replication. Studying human genetic variation in HIV-infected individuals is important to guide a new therapeutic approach.

## INTRODUCTION

Estrogen is one form of the class of steroid hormone that consists of estradiol (E2), estrone and estriol. Estrogen plays a key role in many human physiological processes that range from the development and maintenance of reproductive organs to the regulation of cardiovascular, musculoskeletal, immune, and central nervous system homeostasis.<sup>1-3</sup> Physiological and metabolic effects of estrogens are mediated by the nuclear estrogen receptors (ERs), named estrogen alpha (ER $\alpha$ ) and estrogen receptor beta (ER $\beta$ ), which distinct tissue and cell patterns of expression.<sup>3,4</sup>

The common ERs polymorphisms associated with the expression of the estrogen receptors are PvuII (IVS1–397 T/C; rs2234693) and XbaI (IVS1–351 A/C; rs9340799) that are the most widely studied so far.<sup>5</sup>

It has been shown that ERs receptors have been detected in oral mucosa and salivary glands. The presence of ER mRNA and immunoreactive ER protein suggests that estrogens have a biological role in oral mucosa and salivary glands.<sup>6,7</sup> Throughout the last year, an increasing number of studies have studied the relationships between genetic polymorphisms of PvuII and XbaI and numerous diseases. As the oral mucosa contains estrogen receptors,

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variations in estrogen levels can affect oral mucosa directly or through neural mechanism affect the oral cavity.<sup>8</sup> The previous findings showed that estrogen may be involved in the development or progression of numerous oral diseases, including oral leukoplakia, oral squamous cell carcinoma (OSCC), periodontal disease, xerostomia, burning mouth syndrome (BMS), and temporomandibular joint disorders (TMJD).<sup>9-11</sup>

The amount of attention has been given to the relationship between estrogen and HIV. It has been demonstrated a contribution of estrogen for HIV-1 replication through ER $\alpha$  by augmenting Sp1 DNA-binding and transcriptional activity.<sup>12</sup> In addition, antiretroviral drugs, such as non-nucleoside reverse transcriptase inhibitors, have been showed to be associated with increased levels of estrogen.<sup>13</sup> It is rare to find recent data on estrogen and its receptor in HIV-infected population, especially in HIV-infected children. While children with advanced immunosuppression may frequently develop estrogen-related diseases, including in oral cavity. At present, genotype distribution of ER $\alpha$  gene PvuII and XbaI polymorphisms and their association with HIV-diseases progression has not been reported. It is known well that HIV infection and AIDS among children continues to be a significant problem in developing countries including in Indonesia. This study was conducted to find out an association between ER $\alpha$  gene PvuII and XbaI polymorphisms with CD4<sup>+</sup> T-cell counts as a marker of the degree of immunosuppression in HIV-infected children on highly active antiretroviral therapy (HAART).

## METHODS

### Ethics Statement

The study protocol was reviewed and

approved by Ethical Committee of Faculty of Medicine, Universitas Padjadjaran number 275/UN6.C1.3.2/KEPK/PN/2015. All the parents of children provided written informed consent.

### Study participants

The present study enrolled 34 (thirty-four) HIV-infected children under HAART, aged between 6 and 14 years. All study participants were diagnosed HIV positive at Dr. Hasan Sadikin General Hospital.

### CD4<sup>+</sup> T cell count, genomic DNA extraction and polymorphisms analysis

Blood samples were collected from the study participants and the CD4<sup>+</sup> T-cell counts were determined using the Becton Dickinson (BD) FACS Count system. The genomic DNA was extracted from whole blood using the established protocol for DNA extraction from blood cells. The PvuII and XbaI polymorphisms of ER $\alpha$  were analyzed by polymerase chain reaction restriction fragment length Polymorphism (PCR-RFLP) as described previously.<sup>15</sup>

### Statistical analysis

Data entry and analysis were done using software SPSS version 13. Quantitative data were presented by mean and standard deviation. Qualitative data were presented by frequency distribution. One-way ANOVA and Kruskal-Wallis test were used to detect the mean difference across the two genotypes and the CD4<sup>+</sup> T-cell counts. A confidence interval (CI) level was set to 95% where any output  $p < 0.05$  would be interpreted as an indicator of statistical significant.

## RESULTS

### Characteristics of the study participants

The distributions of characteristics of the thirty-four (34) HIV-infected children on HAART recruited in this study are summarized in Table 1. The gender distributions of the study participants were 16 (47,1 %) males and 18 (52,9 %) females. Their mean age was 9 years (SD  $\pm$  3), ranging from 6 to 14 years. The mean duration of HAART use was 5 years. The mean CD4+ T-cell counts of the study participants was 724 (18 – 1805) cell/mm<sup>3</sup> and 8 participants reported to have CD4 count < 200 cells. Distribution of CD4+ T-cell counts of

the study participants was shown in Tabel 2. The mean of CD4+ T-cell counts of the study participants was cell/mm<sup>3</sup>, and 8 participants reported to have severe immunosuppression (<200/mm<sup>3</sup>) < 200 cell/mm<sup>3</sup>.

### Genotype frequency and its association with the CD4+ T-cell counts

The genotype distributions of the ER $\alpha$  gene PvuII and XbaI polymorphism among HIV-infected children on HAART are shown in Table 3. Genotype PvuII polymorphism was distributed as follows: PP 41,2% (n=14), Pp 26,5% (n=9), pp 32,4% (n=11). The frequency of XbaI polymorphism was XX 35,3% (n=12),

Tabel 1. Characteristics of the study participants

Characteristics	Number of subjects	%
Gender, N (%)		
- Male	16	47,1
- Female	18	52,9
Age (years), Mean $\pm$ SD	9 $\pm$ 3	
Duration of HAART exposure (years), Mean $\pm$ SD	5 $\pm$ 2	
CD4 Absolut	724 (18 – 1805)	
CD4 %	23,72 $\pm$ 10,77	

Table 2. CD4 T-cell counts in relation to the degree of immunosuppression of the study participants.

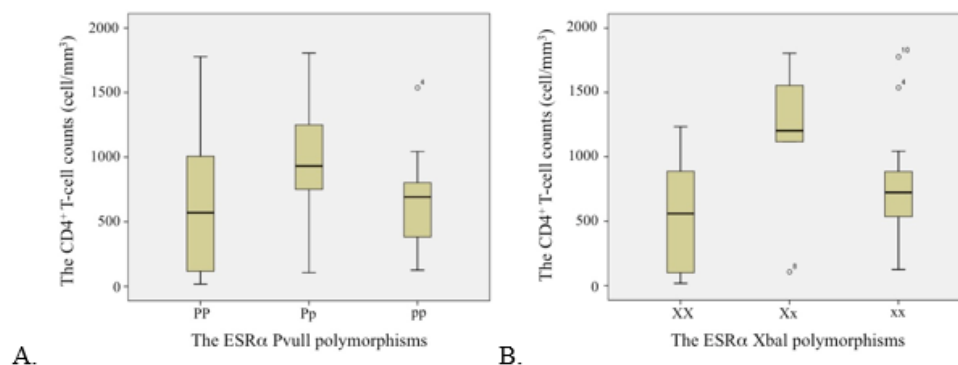
Category	Number of subjects	CD4 T-cell counts Mean/ SD
No significant immunosuppression (>500/mm <sup>3</sup> )	24	946,08 $\pm$ 386,94
Mild immunosuppression (350-499 cell/mm <sup>3</sup> )	1	452
Advanced immunosuppression (200-349 cell /mm <sup>3</sup> )	1	313
Severe immunosuppression (<200 cell /mm <sup>3</sup> )	8	104,63 $\pm$ 45,78

Table 3. Frequency distribution of the ESR $\alpha$  gene PvuII and XbaI polymorphisms in HIV infected children on HAART.

Genotype	Number of subjects	%
Estrogen Receptor PvuII		
- PP	14	41,2
- Pp	9	26,5
- pp	11	32,4
Estrogen Receptor XbaI		
- XX	12	35,3
- Xx	6	17,6
- xx	16	47,1

Table 4. Analysis of the association between ESR $\alpha$  gene PvuII and XbaI polymorphisms and CD4+T-cell counts in HIV-infected children on HAART

The ESR $\alpha$ gene polymorphisms	N (%)	CD T-cell counts Median (range)	p-value
Estrogen Receptor PvuII			
- PP	14 (41,2)	570 (18 – 1777)	0,175
- Pp	9 (26,5)	932 (107 – 1805)	
- Pp	11 (32,4)	691 (126 – 1537)	
Estrogen Receptor XbaI			
- XX	12 (35,3)	560 (18 – 1234)	<b>0,031*</b>
- Xx	6 (17,6)	1203 (107 – 1805)	
- xx	16 (47,1)	724 (126 – 1777)	

Data analysis using Kruskal Wallis test, \* correlation significant  $p < 0,05$ Figure 1. Distribution of the association of the ESR $\alpha$  PvuII polymorphisms (A) and XbaI polymorphisms (B) with CD4+ T-cell counts in HIV-infected children on HAART.

Xx 17,6% (n=6), xx 47,1% (n=16). Analysis for the association of ER $\alpha$  gene Pvull and Xbal polymorphism with CD4<sup>+</sup> T-cell counts were shown in Table 4. It was observed significant association of the Xbal polymorphism with CD4<sup>+</sup> T-cell counts ( $p < 0.05$ ). In subjects with Xx and xx genotype have a higher median of CD4<sup>+</sup> T-cell counts than in subjects with XX genotype. Whereas, there was no significant association were seen for Pvull polymorphism with CD4<sup>+</sup> T-cell counts.

## DISCUSSION

Host genetic factors have been shown to have an important role as a determinant of HIV disease progression.<sup>15</sup> HIV-disease progression in young children is more rapidly than in adults, the effects of host genetic factors on HIV disease progression may be more clearly identified. It has been suggested that host genetic polymorphisms influence the risk of developing numerous diseases in HIV-infected people.<sup>16-18</sup> Estrogene hormone is well known to play an essential role in the growth and development during childhood and estrogen receptors (ERs) mediate much estrogen action.

In this study, we have analyzed the frequency distribution of the ESR $\alpha$  Pvull and Xbal polymorphisms in HIV-infected children on HAART and their association with CD4<sup>+</sup> T-cell counts. Clinical monitoring of infected persons relies on monitoring of CD4<sup>+</sup> T-cell counts and viral load used as a predictor of disease progression and the risk for development of HIV-related complication. Our data showed that PP genotype of ER $\alpha$  gene Pvull polymorphism more frequent than Pp and pp genotype. Whereas, the frequencies of xx and Xx genotype of ER $\alpha$  gene Xbal polymorphism

were higher than XX genotype. Furthermore, Xbal polymorphisms significantly associated with CD4<sup>+</sup> T-cell counts in HIV-infected children on HAART. While, there was no significant association were seen for Pvull polymorphism with CD4<sup>+</sup> T-cell counts. In subjects with Xx and xx genotype have a higher median of CD4<sup>+</sup> T-cell counts than in subjects with XX genotype. It has been reported the direct inhibitory effect of 17 $\beta$ -estradiol, the most bioactive form of estrogen, inhibits HIV replication.<sup>19</sup> Prior studies has been reported that the common ER $\alpha$  polymorphisms and Xbal and Pvull as possible markers for several human diseases and these genotype variations have been implicated in the occurrence of numerous diseases.<sup>20-22</sup>

The host genetic factors may alter the response to antiretroviral. HAART is well-established have an important role to reduce the frequency of opportunistic infections and the number of AIDS-related deaths. However, the adverse effects of HAART use have been reported with the use of all antiretroviral (ARV) drugs.<sup>23</sup> Prior studies demonstrated the influence of antiretroviral therapy on growth hormone therapy in HIV-infected patients. Non-nucleoside reverse transcriptase inhibitors were associated with 17beta-estradiol, the most bioactive form of estrogen.<sup>24</sup> It has also been reported that genetic polymorphisms of estrogen receptors in HIV-infected patients on HAART.<sup>25</sup> Another study suggested that the efavirenz directly induced growth tissue through direct binding and modulation of estrogen receptor.<sup>26</sup>

Estrogen receptors have been detected in oral mucosa and salivary glands, hence it is suggested that estrogens have a biological role in oral mucosa and salivary glands. Prior studies have been reported that some

genetic changes had any effect on oral cavity. These findings could be applicable in oral health therapy. Besides, other factors such as microorganisms, chronic irritation and sex hormones along with underlying genetic factors may also contribute to pathogenesis of oral diseases.<sup>9-11</sup> Genetic polymorphisms of estrogen receptors related to oral disease have not been investigated by any study so far. Recognition of the susceptibility genes for HIV-related oral diseases may also important to initial prevention of disease.

The present study had some limitations. The size of the population studied was relatively small. Therefore, a larger population is required to establish the potential role of the genetic polymorphisms of estrogen receptors as a modifier gene in disease progression in HIV population. During the past decade, the majority of studies investigating the influence of human genetic variation related to HIV disease and treatment response. Much research has established that host genetic factors are important determinants HIV disease progression in children as well as adults. Several anti-HIV-1 gene therapy approaches have been studied, including T-cells gene therapy as co-option for anti-HIV treatment.<sup>27</sup> The impact of human genetic variation on HIV pathogenesis and treatment response is a complex multi factorial phenomenon, which still remains to be fully elucidated and understood.

## CONCLUSIONS

We summarized that the ER $\alpha$  gene XbaI polymorphism significantly associated with CD4+T-cell counts in HIV-infected children on HAART. The relationship between genes determining disease progression and drug response requires much further study focus

on the role of ER $\alpha$  that may help to identify therapeutic or diagnostic markers. HIV-infected children are at increased risk for growth and development disorders and estrogen-associated diseases, which might be influenced by a number of genotypic factors. These findings emphasize the important of studying human genetic variation to guide a new therapeutic approach. A comprehensive study of the molecular genetic variation can be used to predict pharmacological response.

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## CONFLICT OF INTEREST

The authors declare no conflict of interests.

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